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Temperature Influence on Acetyllysine Interaction with Glucose in Model Systems due to Maillard Reaction

K. ANANTH NARAYAN and MICHAEL E. CROSS

ABSTRACT

The interaction between N-α-acetyllysine and glucose was investigated in a dry cellulose matrix (1.8% moisture) at 40, 50 and 60°C and a_w 0.19–0.21. The loss in acetyllysine in 5 days at those temperatures was 22, 54, and 79%, respectively. The degradation data more clearly fit a double exponential function ($Ae^{Bx} + Ce^{Dx}$) than the first order function, Ae^{Bx}. An estimate of the activation energy of 36 kcal/mol and a Q_{10} of 5.5 was computed from second order rate constants for acetyllysine and glucose degradation. The increase in color followed zero order kinetics and indicated an activation energy of 44 kcal/mol for the presumed third phase of the Maillard reaction in the solid state.

Key Words: browning, Maillard-reaction, glucose, acetyllysine, cellulose

INTRODUCTION

PROTEIN QUALITY losses are widely recognized to occur at moisture activities between 0.3 and 0.75 during processing and storage due to the Maillard reaction (Ben-Gara and Zimmerman, 1972; Burvall et al., 1978; Desrosiers et al., 1989; Kato et al., 1986, 1988; Labuza and Saltmarch, 1981; Loncin et al., 1968; Ringe and Love, 1988; Warmbier et al., 1976; Warren and Labuza, 1977). Because of the ease with which absorbance measurements can be made, Maillard browning rather than Maillard protein quality losses has been extensively investigated (Eichner and Karel, 1972; Flink et al., 1974; Hendel et al., 1955; Hsu and Fennema, 1989; Kim et al., 1981; Labuza and Saltmarch, 1981; Mizrahi et al., 1970a, 1970b; Petriella et al., 1988; Stamp and Labuza, 1983). Hence, there are many published reports for kinetic parameters such as Q_{10} and the energy of activation (E_a) for Maillard browning at low a_w (< 0.3) in food systems (Hendel et al., 1955, Flink et al., 1974; Kopelman et al., 1977; Mizrahi, et al., 1970a, 1970b). The water activity (a_w) region of 0.2 to 0.3 is generally considered to be a stable region for foods because the water is tightly bound and not available to facilitate nutrient interactions. Therefore, understandably much of the reported work on food systems has focused in the water activity region 0.33–0.75 to evaluate kinetic parameters of protein or lysine quality loss, particularly E_a and Q_{10}.

Compressed foods and uncompressed food powders used for military subsistence have low a_w (<0.22) and may need to be stored for long periods of time. Not only are data on protein quality loss kinetics at given storage conditions important but also prediction of the temperature sensitivity of protein quality loss at low a_w (<0.22) is useful. Hannan and Lea (1952) reacted N-α-acetyllysine and glucose at 37°C and 20, 40, and 60% relative humidities (R.H.) and showed that 25% of the lysine (as free amino nitrogen) was lost in 3 days at a R.H. of 20%. Burvall et al. (1978) investigated the storage stability of ordinary and lactose-hydrolyzed milk powders at 25°C at a_w of 0.11, 0.22, 0.33 and 0.62. While ordinary milk powder was quite stable at $a_w \leq 0.22$, lactose-hydrolyzed milk powder indicated at 17% loss in lysine at 1 mo storage at 25°C. Similarly, valuable information on the kinetics of lysine loss have been obtained by several investigators (Eichner, 1975; Schnickels et al., 1976; Warmbier et al., 1976; Warren and Labuza, 1977) at one temperature (40, 35, 45, and 35°C respectively) and at one or more a_w (0.23–0.82, 0.63–0.78, 0.52, and 0.3–0.7 respectively). Kaanane and Labuza (1985) have investigated the storage effect of three temperatures of 25, 38, and 45°C on lysine losses in fish flour at a_w 0.33, 0.44, and 0.65. They obtained an E_a of 13.1 kcal/mol for lysine loss at an a_w of 0.33. The data of Ben-Gara and Zimmerman (1972) were analyzed by Labuza and Saltmarch (1981) who have provided E_a values of 14.6 kcal/mol at a_w 0.4 and 19.4 at a_w 0.6 for nonfat dry milk stored at 20, 30 and 40°C. Jokinen et al. (1976) reacted a soybean protein concentrate, glucose, sucrose, starch and cellulose model to temperatures of 80–130°C and a_w 0.33–0.93 and obtained an average E_a of 28.5 kcal/mol for the degradation of lysine. Activation energies for lysine losses in egg noodles of 15.2 ± 9.1 and in pasta of 12.8 ± 5.2 kcal/mol have been reported at a_w 0.44 and 0.49 using storage temperatures of 30–55°C (Chen et al., 1983; Labuza et al., 1982).

In an earlier study, Narayan and Andreotti (1989) investigated the interaction between lysine and glucose in an inert cellulose matrix. Interpretation of the data was complicated by the presence of two reactive groups, the alpha and the epsilon amino groups. In our present investigation, N-α-acetyllysine was reacted with glucose in a cellulose matrix at low a_w in the range 0.19–0.21. The low a_w was selected to simulate the environment in compressed foods and uncompressed food powders used for military subsistence. Determination of acetyllysine was achieved using a reverse phase Pico.Tag® column in a High Performance Liquid Chromatography (HPLC) system.

The specific objectives of this investigation were to investigate the quantitative aspects of acetyllysine availability in a low moisture model, to determine the kinetics of acetyllysine degradation at 40, 50 and 60°C, and to determine E_a and Q_{10} of acetyllysine degradation due to Maillard reaction between acetyllysine and glucose.

MATERIALS & METHODS

Sample preparation

Separate solutions of N-α-acetyllysine (Sigma Chemical Co., St. Louis, MO) and anhydrous glucose (Fisher Scientific Co., Medford, MA) in highly purified deionized Milli Q water (Millipore Corp., Bedford, MA) were prepared. Ten mL volumes of each containing 5.31 mmol were pipetted into several freeze-drying flasks. Dispersions in water containing 15.2g of cellulose (Sigmacell, Type 20, Sigma Chemical Co., St. Louis, MO) were added to each flask, mixed, immediately shell frozen in an alcohol-dry ice bath, and freeze-dried. The dry powders were mixed in a blender. For equilibration, 1g samples of the powder mixture in petri dishes (52 × 15 mm) placed over 50 mL beakers were incubated in separate 237mL Mason jars containing saturated potassium acetate slush at room temperature for 6 days (zero hr samples). They were then stressed at 40, 50, and 60°C over saturated potassium acetate solutions in the Mason jaras for various times (see below) in mechanical convection electrical ovens.

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Table 1—Estimates of parameters for first order mathematical model relating changes in acetyllysine and glucose with time[a]

Temp (°C)	A	[10⁻³]B	$t_{1/2}$ hr OSB[b]	$t_{1/2}$ hr CALC[b]	Deviation[c] %
Mathematical Model[d] $Y = Ae^{BX}$					
Acetyllysine					
40	92.0	−0.527	1000	1300	19
50	93.2	−4.88	100	140	8.6
60	79.7	−18.0	14.5	38	20
Glucose					
40	90.4	−0.600	950	1200	22
50	89.2	−4.18	120	170	12
60	77.6	−9.58	22	72	23

[a] Acetyllysine-glucose-cellulose powders incubated over saturated potassium acetate solution. X = time in hours, Y = acetyllysine or glucose values (in mg/g powder) expressed as percent of zero time values.
[b] OBS = Observed; CALC = Calculated.
[c] Expressed as (Sum of squared residuals) × 100 / (Corrected sum of squares)
[d] First order rate constant equals negative B in $Y = Ae^{BX}$ expressions.

Table 2–Estimates of parameters for double exponential mathematical model relating changes in acetyllysine and glucose with time[a]

Temp (°C)	A	[10⁻³]B	C	[10⁻³]D	$t_{1/2}$ hr OSB[b]	$t_{1/2}$ hr CALC[b]	Deviation[c] %
Mathematical Model $Y = Ae^{BX} + Ce^{DX}$							
Acetyllysine							
40	57.1	−0.129	41.8	−4.17	1000	1100	0.27
50	43.1	−26.6	56.3	−1.60	102	105	0.24
60	37.5	−3.28	58.6	−114	14.5	12.5	1.1
Glucose							
40	58.8	−0.136	41.1	−6.24	950	1200	0.98
50	36.2	−46.2	62.7	−1.83	120	125	0.16
60	48.6	−3.5	50.3	−143	22	17.5	0.64

[a] Acetyllysine-glucose-cellulose powders incubated over saturated potassium acetate solution. X = time in hours, Y = acetyllysine or glucose values (in mg/g powder) expressed as percent of zero time values.
[b] OBS = Observed; CALC = Calculated.
[c] Expressed as (Sum of squared residuals) × 100/(Corrected sum of squares)

At periodic intervals, 0, 2, 4, 8, 12, 24, 48, 96, 144, and 288 hr, the 50 and 60°C samples were withdrawn, cooled, and analyzed for acetyllysine, glucose, Amadori compound and color. For the 40°C incubation, the withdrawal times were 0, 4, 8, 24, 72, 144, 288, 576, 1156, and 4848 hr because of the expected slower overall rate of reaction at 40°C compared with the other temperatures. The 40°C samples were analyzed for reactants and products as were the 50 and 60°C samples. The cellulose had an initial a_w of 0.37 and an initial moisture content of 5.0%. Copper, iron and cobalt were not detected by the Perkin Elmer emission spectrometer. The cellulose was 98% pure (dry weight basis). The bulk (98.9%) of the cellulose particles were < 400 mesh.

Water activity measurement

Water activities were measured using a Beckman Model EEJA3 Instrument. Water activities of the saturated potassium acetate solution were measured at 25, 40 and 50°C. The a_w of the powders was measured at 30°C.

Moisture determination

Drying at 80°C or higher temperature was not possible because of continuous release of Maillard second stage degradation components. Hence, moisture was determined in duplicate for each temperature model by initially desiccating at room temperature over Drierite®, later over phosphorus pentoxide to constant weight.

Glucose

The assay employed was the highly specific coupled enzyme reaction catalyzed by hexokinase and glucose-6-phosphate dehydrogenase. Reagents were purchased from Sigma Chemical Co. (St. Louis, MO). Triplicate analyses of the powders were conducted with 3 separate Milli Q water extracts using 40 mg powder and 2 mL water. Centrifugation at 2000 rpm for 15 min was performed to sediment cellulose. The supernatants were clear and free of any cellulose particles. The reduction of NAD to NADH was determined using a Bausch and Lomb Spectrophotometer at 340 nm. Suitable volumes of 20–60 µL were used for the assays.

Color

Absorbance at 410 nm using a Bausch and Lomb Spectrophotometer of Milli Q extracts of the powders (300 mg and 3 mL water) gave a good indication of the third stage Maillard reaction products, i.e. the brown melanoidin pigments.

Acetyllysine

A Waters Pico.Tag® column was used to quantify acetyllysine after derivatization with phenylisothiocyanate. Triplicate analyses of the powders were conducted using 30 mg of powder and 4 mL of Milli Q as in the procedure used for glucose. The solutions were filtered through 0.45 µm Milex filters (Waters Associates, Milford, MA) to prevent clogging of HPLC column during subsequent analysis. Suitable volumes (25–75 µL) were used for derivatization. Two Model 510 pumps were used to obtain the required gradient with Eluent A (pH 6.4 acetate buffer in 6% (v/v) acetonitrile-water) - Eluent B (60% (v/v) acetonitrile-water). A system interface module (SIM) provided microprocessor-control of the pumps and the Waters autosampler (WISP model 710B). It also served as an A/D converter for the detector (Kratos Spectroflow 773) output and was connected with a DEC 350 computer through an input/output (I/O) interface.

The procedure of Bidlingmeyer, Cohen and Tarvin (1984) for amino acids was used. Under these conditions it was possible to separate the reaction product from the unreacted acetyllysine in a Waters HPLC system (Waters Associates, Milford, MA) with detection at 254 nm.

Curve fitting of data and kinetic analysis

A Hewlett Packard, HP 9000, model 236 desk-top computer was used to perform standard nonlinear regression analysis. As one simple means of judging the overall goodness of fit of these nonlinear mathematical models to the data, the sum of squares of the residuals were expressed as percent of the total sum of squares corrected for the mean (Tables 1 and 2). The rate constants and activation energies were determined by standard procedures (Atkins, 1978; Eyerson, 1981; Frost and Pearson, 1953; Jencks, 1969). These values were obtained from separate regression analysis and not by graphical methods. The probable order of the reaction was determined from t/p vs t plots (Wilkinson, 1961) where p = cumulative fraction of acetyllysine or glucose degraded in time t.

RESULTS & DISCUSSION

Application of a standard nonlinear model to fit the kinetic data

The degradation data for acetyllysine and glucose fit a double exponential expression ($Y = Ae^{BX} + Ce^{DX}$) better than the first order expression ($Y = Ae^{BX}$) where X = time and Y = concentration of reactant expressed as a percent of the zero hr value (Fig. 1 and Tables 1 and 2). Figure 1A suggests that the data did not fit the first-order models. The fit appeared to be better using a double exponential expression (see Fig. 1B and 1C). This was further indicated by the much lower percent deviation for the double (0.2 to 1.1) exponential functions (Table 2) compared with the single (9 to 23) exponential functions (Table 1). From the first order expression (Table 1), the first-order rate constant could be derived; it represents the negative value of the coefficient, B. Unlike linear regression, there are no simple means of judging goodness of fit for standard nonlinear models.

Goodness of fit was based upon: comparison of the sum of squared residuals with the total sum of squares corrected for the mean; examination of residuals and residual plots; and correlation coefficients between observed and computed Y values.

Residual plots revealed no indication of notable distortion. Correlation coefficients between observed and computed Y

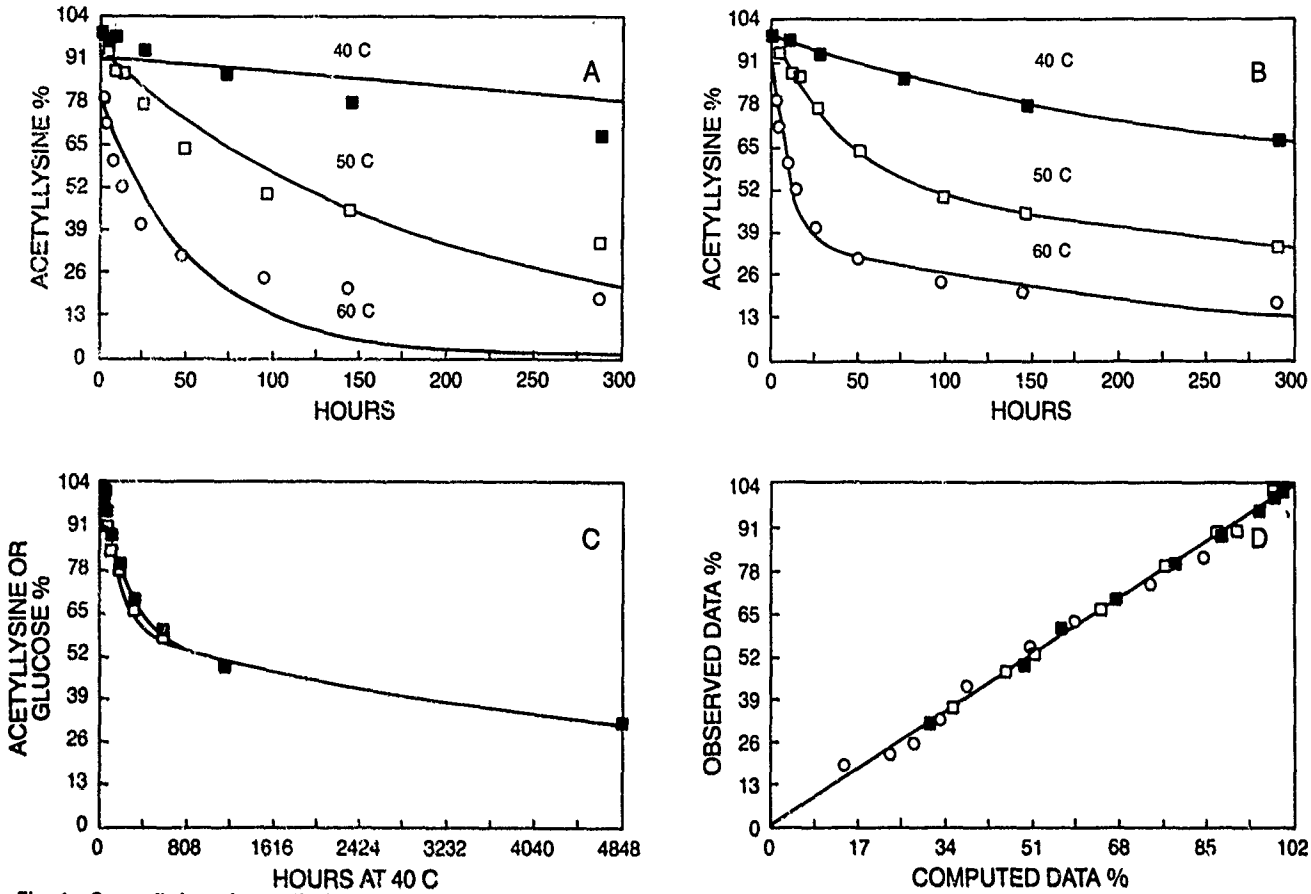

Fig. 1—Curve fitting of acetyllysine and glucose degradation data to exponential functions. Averages of triplicate determinations. The acetyllysine and glucose values (in mg/g powder) are expressed as percent of zero time values. (A) Data fit to the first order exponential equation, $Y = Ae^{BX}$. (B) Data fit to double exponential equations, $Y = Ae^{BX} + Ce^{DX}$. (C) Data fit to double exponential equations, $Y = Ae^{BX} + Ce^{DX}$. Acetyllysine (dark squares) and glucose (open squares) degradation data at 40°C for 0–202 days. (D) Correlation between observed and computed data for acetyllysine loss percent using the double exponential functions at 40 (dark squares), 50 (open squares) and 60°C (open circles).

values provided a third means of testing the goodness of fit. Figure 1D shows excellent correlation ($r>0.99$) between observed and computed values at all temperatures. These data suggest that there was no notable lack of fit of the data to the tentative mathematical models. However, note that the double exponential expression was developed solely to curve fit the data and thus provide empirical predictive information on long term stability and that it was not developed as a mechanistic tool.

Water activity and equilibration

The water activities (measured at 30°C) of the initial freeze-dried powders were in the range 0.043–0.082 and the moisture contents 0.63 to 0.80%. After incubation at room temperature for 6 days, the a_w were in the range 0.242–0.247 and the moisture contents 1.7–1.9% consistent with previous data using a compressed lysine-glucose-cellulose model (Narayan and Andreotti, 1989).

It is always difficult to strike a balance between absolute equilibration (constant weight to ± 1 mg/g sample) and limiting the degradation at zero time. In published studies a variety of equilibration methods have been used. These included 5°C and a_w 0.33–0.65 for 2 wk with a large quantity of whey powder (Saltmarch et al., 1981). Room temperature (ca 21°C) and a_w 0.11–0.65 for 1 wk was used with 200g of whey powder (Kim et al., 1981). At 50°C no equilibration at a_w 0.65 was used for ovalbumin-aldohexose models (Kato et al., 1986, 1988). At 25, 30 or 45°C and a_w 0.11–0.85 for 2 to 3 wk was used for fish flour (Labuza et al., 1985). At 40°C, and a_w of 0.23 to 0.82 apparently no equilibration was used for a lysine-glucose-Avicel model system (Eichner, 1975). Infusion of humidified air at 40°C was used with subsequent blending of dry and moistened whey protein concentrates (Hsu and Fennema, 1989). Temperature 25°C and a_w of 0.11 to 0.62 were used with regular and lactose-hydrolyzed milk powder for 1 to 4 days (Burvall et al., 1978).

The attainment of a_w in the range 0.242–0.247 in our experiments suggested that equilibration had been reached. Further, the low initial a_w and the low moisture content of the initial freeze-dried models suggested that equilibration occurred along the adsorption path rather than the desorption path of the hystersis loop.

The water activity of saturated potassium acetate solution is influenced by temperature. The observed a_w values at 40 and 50°C were both 0.21. Since the instrument could not be operated above 50°C, the a_w at 60°C was estimated in four different ways. Using Raoult's Law and a computed activity coefficient at a_w 40 and 50°C gave a value of 0.19. Extrapolation of the data of Greenspan (1977) from 5 to 30°C gave a_w values of 0.21, 0.20 and 0.19 at 40, 50 and 60°C respectively for a saturated potassium acetate solution. Regression analysis relating Ln a_w with 1/T (where T is °K) of our data at 30, 40, and 50°C gave an a_w of 0.20 at 60°C. This a_w was somewhat higher than predicted (a_w = 0.175 at 60°C) by the regression equation for potassium acetate by Labuza et al. (1985). In our experiments, the assumption was made that the models equilibrated to the a_w of the salt solutions at the three temperatures following 6 day incubation at room temperature.

The use of small Mason Jars appeared to provide rapid equil-

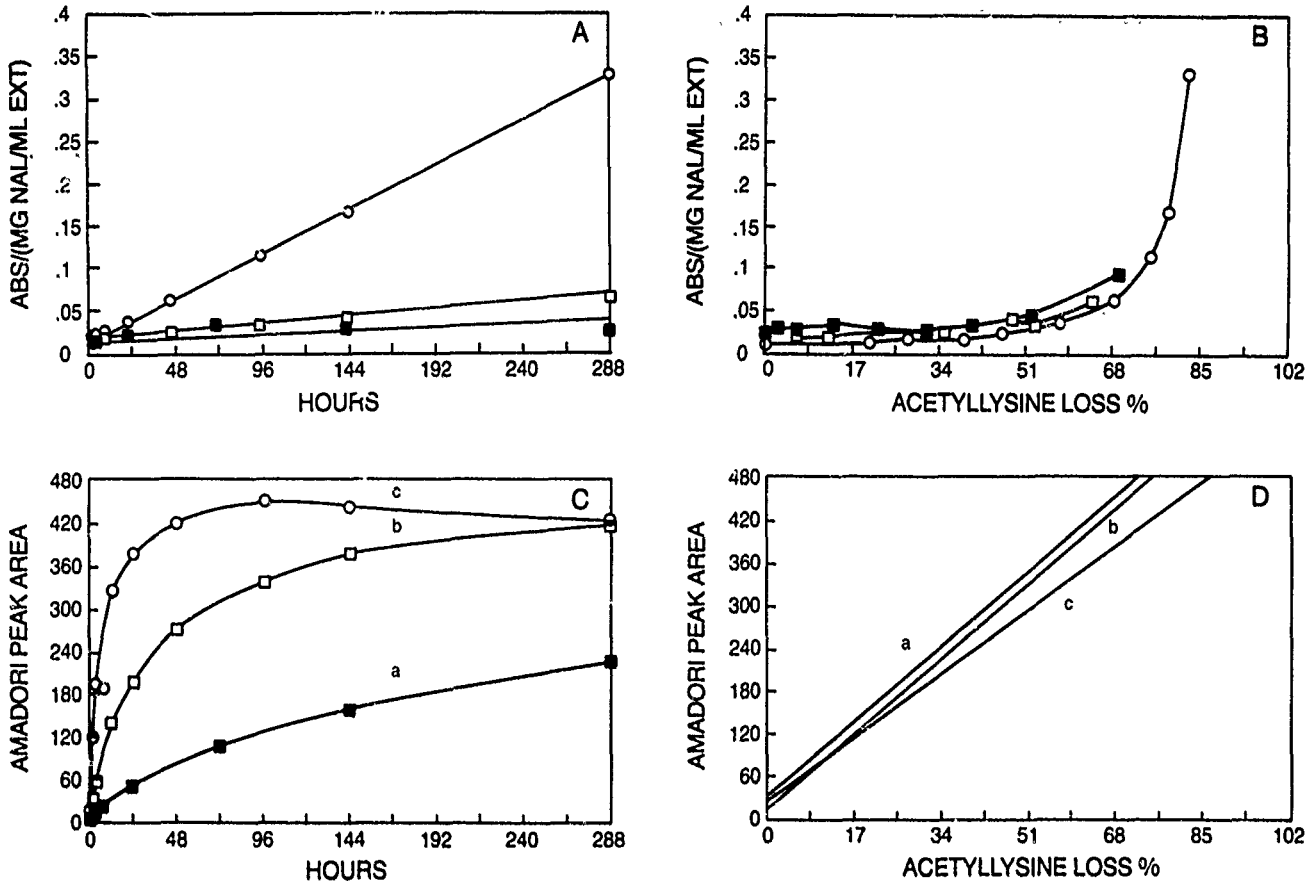

Fig. 2—Quantitative aspects of the formation of brown pigments and of Amadori compound. The data at 40, 50, and 60°C are represented by either dark squares and/or curve a; open squares and/or curve b; and open circles and/or curve c, respectively. (A) The influence of time and temperature on the formation of brown pigments (absorbance at 410 nm/mg acetyllysine per mL of extract at zero hr). (B) Correlation between color and the loss in acetyllysine. (C) Influence of time and temperature on the formation of Amadori compounds expressed as [10^2] peak area units/mg acetyllysine per mL of extract at zero hr. (D) Correlation between the increase in peak area of the Amadori compound and the decrease in acetyllysine.

ibration for both powders and compressed bars (Narayan and Andreotti, 1989) because of the greatly reduced headspace as compared with the conventional desiccator method. Further, it was possible to withdraw each sample (i.e. each Jar) at a precise time without affecting the a_w or temperature of other samples.

Brown pigments

The changes in absorbance at 410 nm were linear with time at all temperatures (Fig. 2A). As with the lysine-glucose model reported earlier (Narayan and Andreotti, 1989), no simple linear relationship was found between color and loss in acetyllysine (Fig. 2B). The large effect of temperature seen here (Fig. 2A) and reported before (Narayan and Andreotti, 1989) may have been due to the low a_w of the model. Activation energies for Maillard browning are known to increase with a decrease in a_w (Mizrahi et al., 1970b; Hendel et al., 1955).

Amadori compounds

A peak with retention time 3.4 min was observed during reactions at 40, 50 and 60°C. During the HPLC analysis for acetyllysine, the area of the peak was recorded and related to changes in peak areas for acetyllysine at different reaction times. In order for the unknown compound (peak at 3.4 min) to be considered as an Amadori compound, it had to meet several minimum requirements. The compound should have a reactive group capable of coupling with phenylisothiocyanate. The phenylthiocarbamyl derivative should be significantly polar and should elute early in the run on a reversed phase C-18 column. The compound should be present in insignificant amounts in the early stages of the reaction. Its concentration should increase with time coincident with the decrease in concentration of the reactant. Since the compound which eluted at 3.4 min met these criteria, it was tentatively identified as the Amadori compound.

The concentration of the compound increased exponentially with time (Fig 2C) and correlated well ($r=0.98$, at all temperatures) with the decrease in acetyllysine, as would be expected with formation of an Amadori compound (Fig. 2D). These data are similar to the high degree of correlation observed in a lysine-glucose-cellulose model (Narayan and Andreotti, 1989) between lysine loss and the formation of Amadori compounds estimated indirectly by furosine.

Order of the reaction

Applicability of kinetic theory to low a_w solid systems. Evidently the reaction in low a_w solid matrices takes place under totally nonideal conditions which are far different from reactions in homogeneous solutions (Frost and Pearson, 1953). In our present study, we have a heterogeneous system and the reactants may not always be in close proximity, especially during the terminal stages of the reaction. High diffusional resistance associated with a low a_w low moisture system, can seriously impede mobility of the reactants. Although here we are primarily concerned with the disappearance of acetyllysine or glucose during the first phase, the Maillard reaction is complex and multistage. As a result, there could be selective in-

hibition spots. While the straight-forward application of the classical chemical kinetic theory to our model may be subject to question, it has been applied in the food area (Labuza and Saltmarch, 1981; Lea and Hannan, 1949; Saguy and Karel, 1980; Wolf et al., 1977, 1981) and has provided considerable information on shelf life of foods.

Previous studies on lysine loss kinetics. Lenz and Lund (1980) have stated that a first order model adequately described degradation of food components. It is widely accepted that lysine loss in food systems undergoing Maillard degradation follows first order kinetics (Labuza and Saltmarch, 1981). That is based upon numerous studies where, at least up to 50% loss, the semilog plots of concentration vs time were linear (Lee et al., 1984; Labuza and Massaro, 1990; Warmbier et al., 1976; Warren and Labuza, 1977; Wolf et al., 1977).

Wolf et al. (1977) concluded that the hypothesis for a first order assumption for the lysine loss in a soybean protein concentrate, glucose, Avicel model could not be rejected even at 50% confidence level. Additional studies by Wolf et al. (1981) have further confirmed that postulate. The precise ratio of glucose/lysine was not reported in those experiments. Lee et al. (1984) evaluated the kinetics of lysine degradation for the lysine-glucose Maillard reaction in 100% methanol using a very large excess of glucose and observed pseudo-first order kinetics for lysine loss up to only 70%. A noticeable curvature of the semilog plot was noticed after 70% loss. Lea and Hannan (1949) assumed zero order kinetics for the initial reaction between casein and glucose at 70% relative humidity. Jokinen et al. (1976) have noted that zero order kinetics was applicable for the initial lysine loss in a model system containing soybean protein, glucose, sucrose, starch and Avicel processed between 80 and 130°C.

Labuza and Massaro (1990) investigated lysine loss in a model total parenteral nutrition (TPN) solution at a pH of about 3.8. They have suggested that the complex kinetics they observed could be explained on the basis of a Bodenstein steady state approximation. They proposed a balance between the rate of lysine regenerated from the Amadori product and the rate of degradation of lysine through the normal first phase Maillard reaction between lysine and glucose. This is an interesting possibility and would certainly account for anomalies in the first order plots observed by previous investigators. While it is well known that up to 50% of the lysine is recovered upon hydrolysis of the Amadori compound using concentrated acids, (Hodge, 1953; Hurrel and Carpenter, 1981; Finot et al., 1981) data on regeneration of lysine at normal food pH (5–6) are not available. Investigations on the influence of time, temperature, pH and water activity on freshly synthesized fructose-lysine may help to test and extend this hyporthesis.

Reaction kinetics and molecularity. In a first order reaction, the rate is proportional to concentration of a reactant. In a second order reaction, the rate is proportional to the product of the concentrations of two reactants. A second order reaction may appear to behave like a first order reaction when there is a very large excess of one of the reactants. Although molecularity and order are not synonymous, they are often coincidental (Glasstone, 1961). Further, a bimolecular reaction is usually second order (Frost and Pearson, 1953). Thus, it is easy to conceive that the thermal degradation of thiamin would follow first order kinetics. For the first phase of the Maillard reaction between lysine and glucose, teleologically we would theorize that the reaction rate would be dependent upon the concentration of the 2 reacting species. In the past, Tsao et al. (1978) made the explicit assumption that lysine loss followed a monomolecular reaction. On the basis of partial linearities of the first order plots, other investigators have concluded that lysine loss followed first order kinetics.

Determination of the kinetic order of the reaction. Our study is one of the few investigations to follow kinetics of the lysine-glucose reaction where the glucose/acetyllysine ratio at zero time was set at 1. The semilog plot of concentration vs time was a straight line only up to ≤ 50% loss and confirmed numerous other published data described above. In the studies where the glucose/lysine ratio was kept at 2.3, nonlinearity of the first order plots was also observed (Narayan and Andreotti, 1989).

In our present study, the second-order rate constants were obtained by plotting the reciprocal of the concentrations vs time at the three temperatures (Fig. 3A). The correlation coefficients of the linear expressions were 0.95, 0.98 and 0.93, at 40, 50, and 60°C, respectively. The lack of better linearity, particularly at 40, and 60°C, of these reciprocal plots posed the question whether the reaction followed second order kinetics or some other kinetics at all temperatures. Wilkinson plots (1961) were therefore constructed for all temperatures by plotting t/p vs t where p = cumulative fraction of reactant degraded at time = t. Wilkinson has shown that the order of a reaction (zero, first, second or third) could be determined from the slope of a single plot. On the basis of these plots (Fig. 3B), a reaction order of 2.2 to 2.6 was estimated for acetyllysine degradation at the three temperatures. This led to the conclusion that the reaction was more appropriately described by a second order rate expression than by a first order rate expression at all three temperatures.

The advantage of a Wilkinson plot is that it permits a simple differentiation between first-order and second-order reactions on the basis of initial data points in an experiment (Espenson, 1981). Furthermore, Espenson has convincingly demonstrated that if the data from a second order reaction are plotted as a first order plot (Ln concentration vs time) noticeable curvature would be observed beyond approximately 50% reaction. It would be imprudent to conclude that every nonlinear first order plot implies a second order kinetics. However, further tests such as fractional time approach, Wilkinson plots and statistical testing of rate constants to determine the order of the reaction should not be excluded (Espenson, 1981; Frost and Pearson, 1953; Jencks, 1969; Wilkinson, 1961; Wolf et al., 1981).

Estimation of activation energy and Q_{10}

In a previous study, Narayan and Andreotti (1989) evaluated the interaction between lysine and glucose in a cellulose matrix. The present model was less complex since acetyllysine has only one free amino group and is therefore comparable to a protein-reducing sugar model. Using second order rate constants, the E_a and Q_{10} were coincidentally 36 kcal/mol and 5.5 respectively for the degradation of both acetyllysine and glucose (Fig. 3C).

The estimated E_a of 36 kcal/mol from the present study was lower than the value of 57 kcal/mol observed for the lysine-glucose-cellulose model and is attributable to the greater sensitivity to temperature of the lysine-glucose model. The first order rate constants of 0.52×10^{-3} hr^{-1} and 1.89×10^{-3} hr^{-1} at 40°C for the acetyllysine and lysine degradation, respectively, gave an indication of the greater reactivity of lysine at 40°C. Similarly, the first-order rate constants at 50 and 60°C for the acetyllysine-glucose model were considerably lower than those observed for the lysine-glucose model (5.0×10^{-3} hr^{-1} and 23×10^{-3} hr^{-1}, respectively, vs 54×10^{-3} hr^{-1} and 472×10^{-3} hr^{-1}, respectively).

Activation energy values have also been computed from first order fitted equations (Table 1) for acetyllysine and glucose degradations. Those values were for acetyllysine 37 and glucose 29 kcal/mol. The values are close to that obtained from actyllysine loss using second order rate constants. For glucose loss, the E_a value was not substantially different from that computed with second order rate constants.

Comparison of computed E_a value with published values. With respect to losses in protein quality and available lysine, Lea and Hannan (1949) have obtained an E_a of 29 kcal/mol in a casein-glucose food system equilibrated to 70% R.H. and stressed between 0 to 70°C. Jokinen et al. (1976) reported an

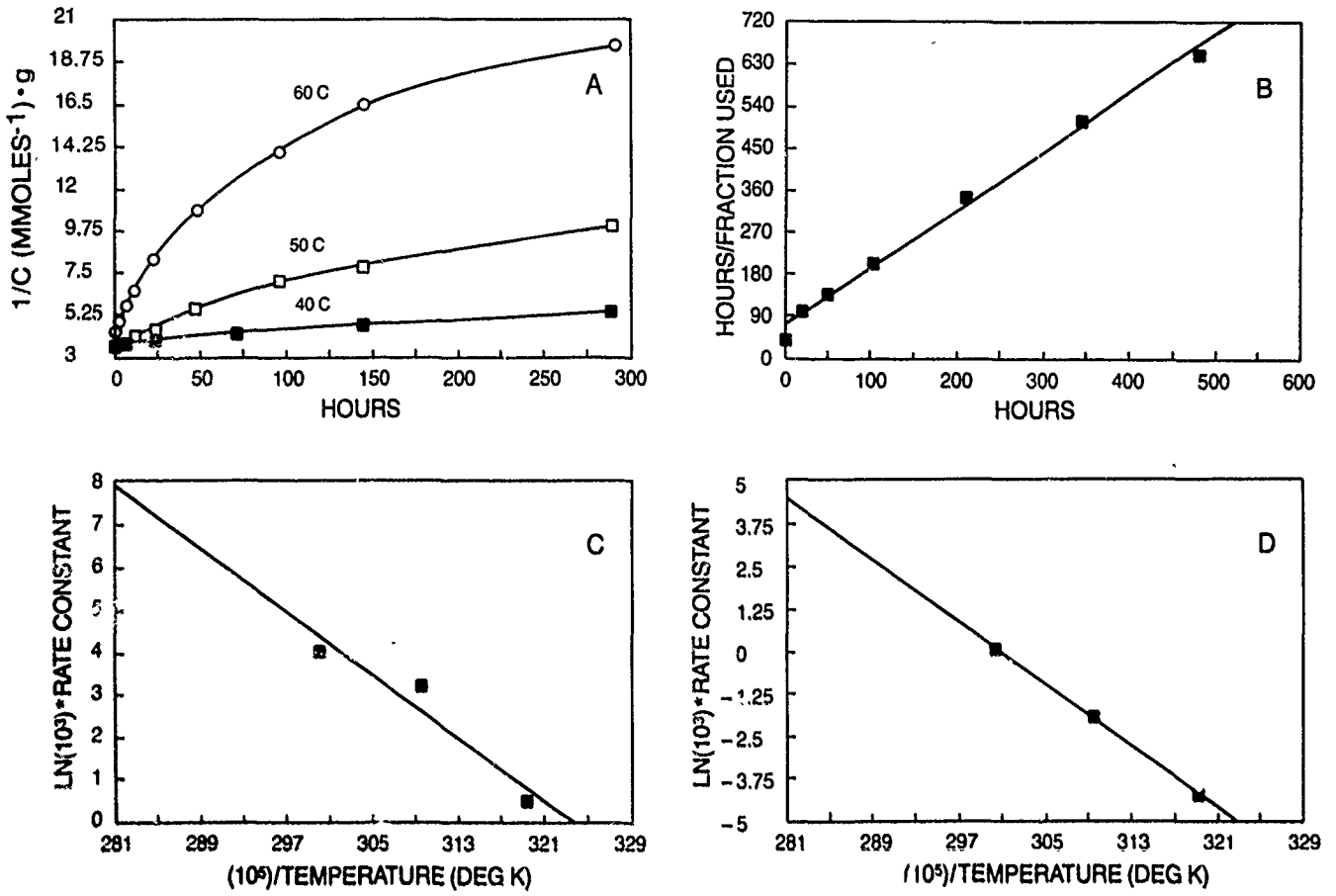

Fig. 3—Determination of order of the reaction between acetyllysine and glucose, and the determination of the E_a for acetyllysine degradation and Maillard browning. (A) Second order plots for acetyllysine degradation at 40, 50, and 60°C: 1/c plotted against time. (B) Wilkinson plot t/p vs t of data obtained at 50°C for lysine degradation. p = fraction completed in time t. (C) Arrhenius plot for acetyllysine loss at 40, 50, and 60°C. (D) Arrhenius plot for Maillard browning at 40, 50, and 60°C.

E_a of 28.5 kcal/mol for a soybean-glucose model system stressed at 30 to 80°C and a_w of 0.33 to 0.93. Similar E_a values in the range 25–37.5 kcal/mol has been reported by other workers (Carpenter et al., 1962; Taira et al., 1966) in two food systems (herring press cake and soybean meal, respectively). Our value of 36 kcal/mol (Fig. 3C) was in the range of those studies. However, low E_a values in the range of 5 to 19 kcal/mol have also been reported (Ben-Gara and Zimmerman, 1972; Chen et. al., 1983; Kaanane and Labuza, 1985; Labuza et al., 1982; Tsao et al., 1978) in several food systems (nonfat mild powder, pasta, fish flour, egg noodles, and fortified rice meal respectively). The low E_a values in the range 5–13 kcal/mol for fish flour stored in open and sealed systems at 25, 38 and 45°C at three a_w of 0.33, 0.44, and 0.65 have been attributed to a free radical induced reaction, rather than to the Maillard reaction (Kaanane and Labuza, 1985).

The low E_a value of 12.5 kcal/mol obtained by Tsao et al. (1978) for fortified rice meal has been rationalized on the basis of the physical structure and the high temperatures used (Labuza and Saltmarch, 1981). Further, it is recognized the trinitrobenzene sulfonic acid method used vastly overestimates available lysine (Hurrel and Carpenter, 1981; Mauron, 1981). The low E_a values for lysine losses in pasta and egg noodles may, in part, be related to the reliability of the rate constants at low temperatures where less than 25% lysine loss occurred.

Maillard browning. The linear responses for color with time (r=0.98 to 0.999) in our present experiments have permitted us to compute an E_a of 44 kcal/mol from zero order rate constants for Maillard browning (Fig. 3D). As an explanation for the wide difference in kinetics between lysine loss and browning, note that pigment formation is not part of the main reaction between acetyllysine and glucose but essentially takes place during the third phase of the Maillard reaction.

Stamp and Labuza (1983) estimated an E_a of 15.5 kcal/mol for Maillard browning in solution due to reaction of glycine with glucose at a_w 0.80. Published values of E_a for Maillard browning vary over a wide range from 16 to 40 kcal/mol and appear to be dependent upon a_w. The high E_a of 44 kcal/mol we observed was consistent with previous observations (Hendel et al., 1955; Mizrahi et al., 1970b) that E_a for browning is inversely related to the a_w.

CONCLUSIONS

THE ACETYLLYSINE-GLUCOSE MODEL is comparable to a protein-glucose model because in both cases, only the ϵ-amino groups are free for interaction. The protein model requires enzymatic digestion to liberate the amino acids. In the acetyllysine model, a simple extraction followed by derivatization at room temperature for 20 min is all that is necessary to follow the degradation of acetyllysine. It is of considerable interest that 22 and 32% of the acetyllysine were lost in 6 and 12 days, respectively, in this model under relatively moderate conditions (a_w = 0.21 and 40°C). Additionally, as much as 69% was lost in less than 7 mo. Note, however that the reducing sugar was present at an equimol level with respect to acetyllysine, a situation that may not prevail in most foods. Dry foods (such as milk chocolate, yogurt products, dried fruits, milk powders, dairy cream products, egg powders, pancake mixes, cake mixes and biscuit mixes) containing proteins and/or amino acids and reducing sugars may be particularly vulnerable to adverse changes in protein quality and consequently

adversely influence shelf life and consumer acceptance. Further, our observations suggest that these protein quality losses may occur at a greater rate than recognized on the basis of generally accepted water activity-quality loss curves.

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